IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Richard L. Moss, et al.
Application No.:	10/748,354
For:	TRANSGENIC MODEL FOR MYOCARDIAL FUNCTION
Filed:	December 30, 2003
Group Art Unit:	1632
Examiner:	SGAGIAS, Magdalene K.
Attorney Docket No.:	054030-0045

AFFIDAVIT UNDER RULE 1.132

I, Richard L. Moss, the undersigned, declare as follows:

All statements made herein are true to the best of my knowledge, or if made upon information and belief are believed to be true.

I received a Ph.D. in Physiology & Biophysics from the University of Vermont in 1975 and have been a professor of Physiology at the University of Wisconsin-Madison since 1979 and Chair of the department since 1988. My areas of expertise are the contractile processes of the heart and alterations therefrom that result in such diseases as heart failure and the mechanisms by which calcium, various physical factors and signaling pathway regulate mycocardial performance. I have over 120 peer review publications in these fields.

Part of my research investigates the molecular mechanisms determining the work capabilities of myosin molecules studying the kinetics of nucleotide turnover by myosin. Different myosin isoforms have widely varying work rates, which seem to contribute to variable myocardial function in different regions of the heart. My research of these mechanisms features systematic mutations of the myosin molecule and expression of myosin in animal models.

I am a principal inventor on the research leading to the above captioned patent application. Accordingly, I am completely familiar with the subject matter of this patent application including the Claims. Under my supervision and/or control the following described experiments were performed to evaluate the unique physiological properties of a transgenic

mouse expressing a transgene comprising a S342G mutation in loop 1of the cardiac alpha myosin heavy chain.

The methods for construction the transgenic mouse expressing the S342G mutant transgene are explicitly described in the above referenced patent specification. For example, general methodologies begin on page 7, line 2 of the patent application with the specific methods used for the creation of the transgenic mouse beginning on page 16 of the patent application.

Example II, given in the application, beginning on page 42 is specifically directed to the substitution of loop 1 IMD of mouse α cardiac myosin. Following a review of the physiology of species components to myosin function, protocols for the molecular biological techniques used in cloning the mouse α myosin heavy chain (MHC) gene are given on page 51. The generation of the transgenic animal expressing the mutant MHC is then describe in the section beginning on page 61. Other examples of the generation of transgenic mouse lines are given on page 64.

The success of the techniques recited in the above passages is measured by the successful production of the claimed transgenic mouse at the University of Wisconsin-Madison, Transgenic Animal Facility. Genetic analysis of the mouse shows that it is homozygous for the S342G mutation as shown in the attached **Figure 1**. Further, physiological studies of the mouse show that it has a decreased rate of force redevelopment and a greater force measured at 32 ± 5 mN/mm^2 for the transgenic animal compared to 23 ± 2 for the wild type animals, see, **Figures 2A-B** and **Table A**. Not surprisingly, while the rate of force redevelopment was decreased and the absolute force generated was increased, the heart rate of the transgenic animal was decreased at 550 for the transgenic animal compared to 606 for the wild type, see, **Table B**. These changes in cardiovascular parameters correspond to those described and claimed in the above captioned patent application.

Accordingly, the successful production of a transgenic mouse by the methods described in this patent application is commensurate with the data derived from the transgenic mouse prepared as described in the application.

This declaration is made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 USC Sec. 1001, and may jeopardize the validity of the subject patent application or ay patent issuing thereon.

Dated: July 10, 2006

By: Richard L. Moss
Richard L. Moss

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Figure 1.

S341G KO PCR

This photograph represents results obtained from PCR-based genotyping designed to identify the mutated S341G gene in our transgenic mice. The white band in each column is mutant DNA, amplified out of our S341G transgenic mice. There is no band in the lane for the 13th sample because it is a negative water control and run only to check that reagents are not contaminated. There are multiple white bands in the first lane because it is a DNA standard, and does not represent DNA from our colony. Every other column has one white band in it, signifying that each of these animals carries our mutant gene.

S341G WT PCR

This image represents results obtained from a second PCR-based genotyping experiment on the same 13 samples as the KO PCR. This experiment was designed to identify the healthy endogenous S341G gene, also called a non-transgenic gene, in our mice. There are no bands in any lane except the positive control lane (DNA we know does carry the gene, and meant to test that the reagents are working). In other words, these two pictures, when taken together, show that we have bred the S341G heterozygous mouse to create the S341G homozygote mutant strain. These animals carry two copies of our mutant S341G DNA and no healthy, wild-type DNA, meaning they are targeted knock-out mice at the S341G locus.

Figures 2A and **B.**

Are graphs plotting the rate of force redevelopment (s^{-1}) as a function of calcium potential (pCa) and normalized force as a function of time. These experiments were performed to assess the turnover kinetics of the mutant myosin by determining the rate of force development in myocardium from the S341G transgenic mice. The experiment employs the following protocol: permeabilized myocardium is activated to varying degrees by varying the amount of activating Ca2+ in the muscle bath; once steady force is achieved, the preparation is rapidly released and restretched, thereby reducing force to zero; the rate constant of force redevelopment (k_{tr}), which is a direct measure of myosin turnover kinetics, is then estimated by fitting an exponential to the time course of force redevelopment (Figure 2B). As disclosed in the present specification, the substitution at residue 341 of myosin heavy chain slowed the rate of rise of force approximately 30% (Figure 2A), indicating that this residue is a primary determinant of myosin turnover kinetics in the heart. Furthermore, the inventors' previous finding that mouse is unique in having a serine at residue 341 of cardiac myosin and that all other myosins in other species have a glycine at 341 indicates that this substitution is responsible for the faster turnover kinetics of mouse cardiac myosin.

Table A.

Is a table showing the absolute force generated by the S341G transgenic and the wild type control.

Table B.

Is a table showing the heart rate of the S341G transgenic and the wild type control.

Figure 1A

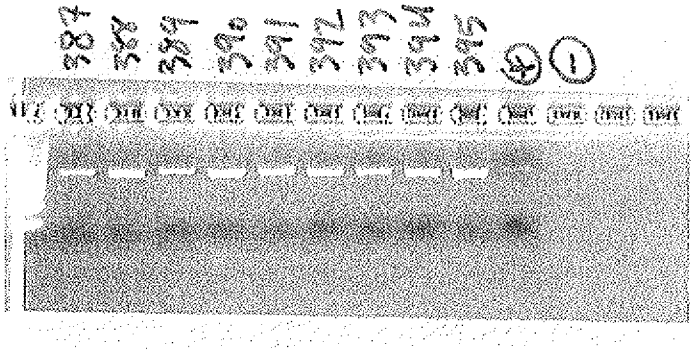

Figure 1B

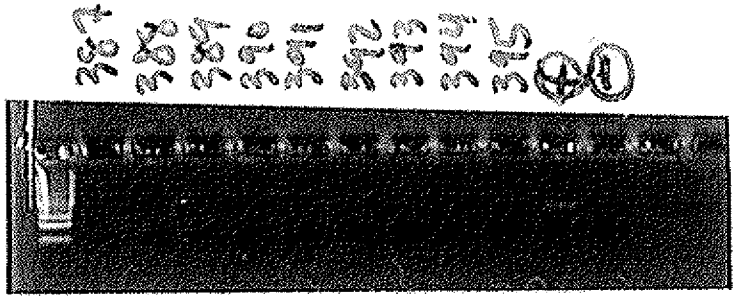

Figure 2A

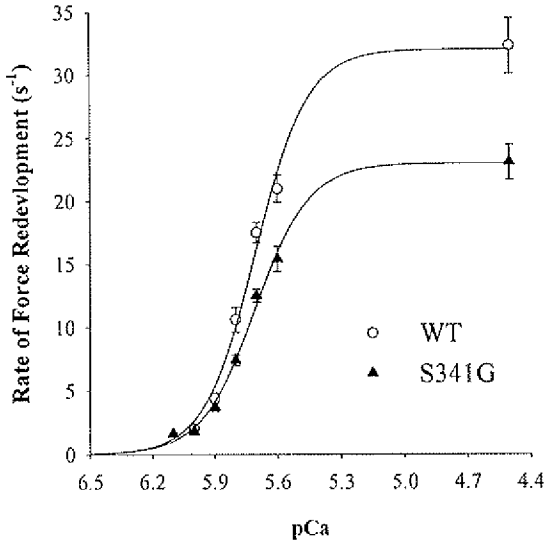

Figure 2B

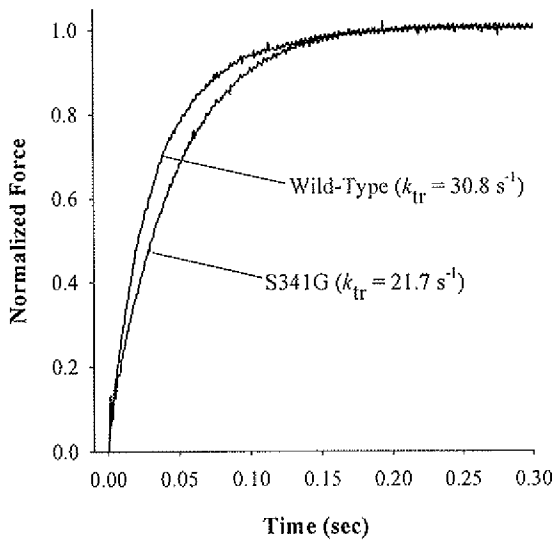

Table A

Animal	Force (mN/mm^2)
Wild Type	32±5 (n=12)
S342G Transgenic	23±2 (n=8)

Table B

Animal	Heart Rate (beat/min)
Wild Type	606
S342G Transgenic	550